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CONTENTS

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The Agricultural Chemical Society of Japan.

President : Umetarō SUZUKI.

The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2. No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japaneses texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

Editor : Umetarō SUZUKI.

Associate Editors : Kakuji GOTŌ and Yoshihiko MATSUYAMA.

ON THE NUTRITIVE VALUE OF SYNTHETIC FATS CONTAINING OXY-FATTY ACIDS.

By Junichi Ozaki.

Biochemical Laboratory, Faculty of Agriculture, Tokyo Imperial University.

(Received June 25th., 1926.)

In continuation to the previous report[1] the author carried out the feeding experiment with synthetic fats containing oxy-fatty acids to compare their nutritive value with other kinds of fats. Oxy fatty acids, except ricinoleic acid, occur in natural fats only in negligible quantity, and so they seem to play only a subordinate rôle in the animal nutrition. But, according to Knoop, β–oxy acids are formed as the intermediate products of the succesive degradation of long fatty acid chains. Further, the fate of other oxy acids having one or more OH group at different position has never been studied thoroughly. The feeding experiments with these fats will therefore contribute something to the knowledge of the fat metabolism in the animal body.

The author has prepared 13 kinds of oxy acids, which were carefully purified and their melting–and boiling-points as well as molecular weights thoroughly examined.

The preparation of triglycerides from these oxy acids was carried out by means of Twitchell's reagent as described in the first report.[1] But, lactin, dioxyundecylin, dioxystearin and trioxystearin could not be prepared by this method, probably these oxy acids being dehydrated by Twitchell's reagent and subsequently decomposed on heating ; and so they were previously acetylated and converted into acetylated glycerides.

Beside these synthetic fats, the author used also the mixture of these oxy acids and glycerine. Furthermore, the nutritive value of ricinolein, acetyl ricinolein and ricinoelaidin were compared one another. The method of feeding used here was exactly the same with that of the former experiment,[1] i. e. the young rats were first fed with a limitted quantity of basal diet (9 grams per day per rat), and when the growth was stopped they were supplied with the test diet containing 5, 10 and 20 % of the fats under examination to the basal diet, respectively, and the growth induced thereby were compared each other. These experiments were carried out in the same season and possibly under the same condition.

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(1) This Journal, Vol. II. No. 1, 1926.

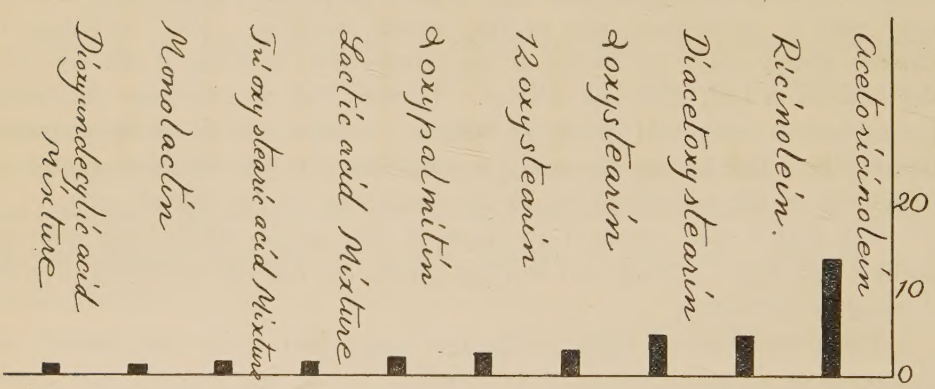
The results thus obtained were as follows:

(1) The growths induced by adding 5% of each fat to the basal diet were shown in the following order:

1.	Acetylricinolein	13.5g.	2.	Ricinolein	4.5g.
3.	Diacetoxystearin	4.5g.	4.	α-Oxystearin	3g.
5.	1 2-Oxystearin	2.5g.	6.	α-Oxypalmitin	2g.
7.	Lactic acid & Glycerin	1.5g.	8.	Trioxystearic acid & Glycerin	1.5g.
9.	Monolactin	1g.	10.	Dioxyundecylic acid & Glycerin	1.0g.
11.	Triacetoxystearin*		12.	Dioxystearic acid and Glycerin*	
13.	Diacetoxyundecylin*		14.	α-Oxyheptylin*	
15.	α-Oxymyristin*		16.	Ricinoelaidin*	

FIG. 1.

Showing the increase of body weight after adding 5 % of Sample to the basal diet,



(2) By adding 10% of each fat:

1.	Acetylricinolein	58.5g.	2.	1 2-Oxystearin	24.5g.
3.	Dioxystearic acid & Glycerin	19.0g.	4.	Monolactin	19.0g.
5.	Diacetoxystearin	18.5g.	6.	α-Oxystearin	17.0g.
7.	α-Oxystearic acid & Glycerin	7.0g.	8.	α-Oxypalmitin	4.0g.
9.	Ricinolein	3.0g.	10.	Dioxyundecylic acid & Glycerin*	
11.	Trioxystearic acid & Glycerin*		12.	Ricinoelaidin*	
13.	Triacetoxystearin*		14.	Diacetoxyundecylin*	
15.	Lactic acid & Glycerin*		16.	α-Oxyheptylin*	
17.	α-Oxymyristin*				

(3) By adding 20% of each fat:

1.	Acetyl ricinolein	63.0g.	2.	Dioxystearic acid & Glycerin	18.0g.
3.	1 2-Oxystearin	16.5g.	4.	Diacetoxystearin	4.5g.
5.	α-Oxystearin	2.5g.			

FIG. 2.

Showing the increase of body weight after adding 10% of Sample to the basal diet.

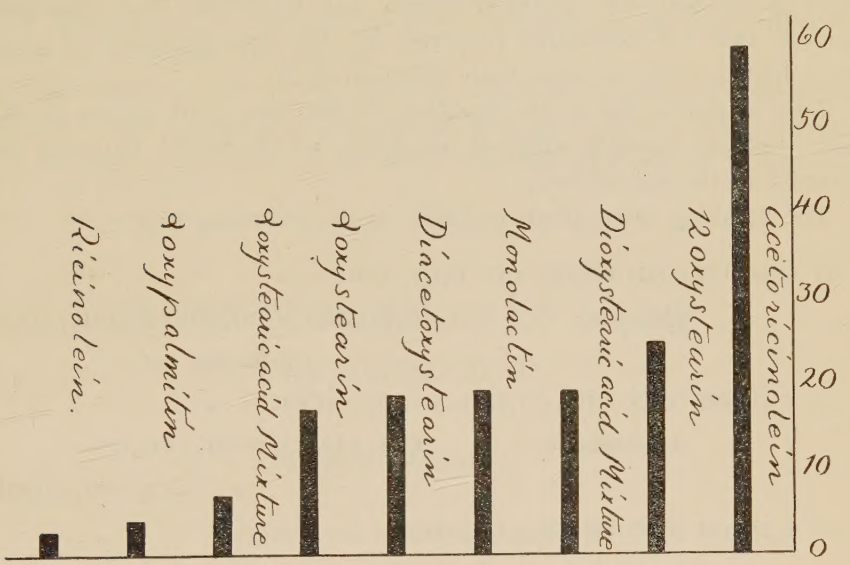
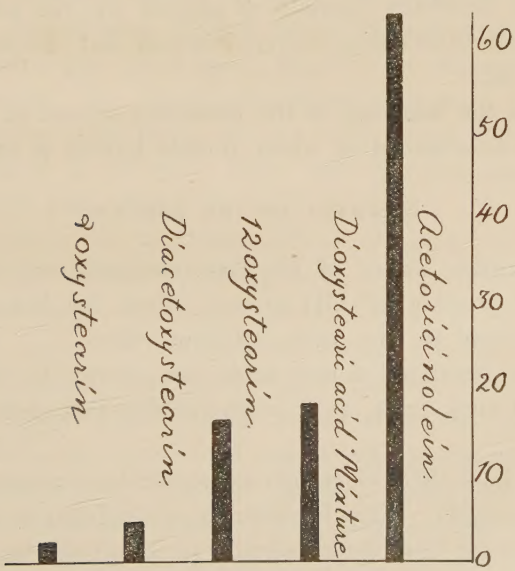


FIG. 3.

Showing the increase of body weight after adding 20% of Sample to the basal diet.



In the above tables, those fats assigned with asteriks* were found to have no nutritive value, and some of them being decidedly noxious of special interest is the fact that acetyl ricinolein was far better than ricinolein and moreover that 1 2-oxystearin prepared by the hydrogenation of ricinolein gave a higher nutritive value than ricinoleiń itself.

This might be due to the breaking of the fatty acid chains at different points, yielding thereby different products, which would naturally behave differently in the animal body.

The breaking takes place probably in the following way :-

1) $C_6H_{13}CH(OH)CH_2CH:CH\cdot(CH_2)_7COOH \longrightarrow$

Ricinolein

$C_6H_{13}CHO + CH_3CH:CH\cdot(CH_2)_7COOH$

Undecyleic acid.

2) $C_6H_{13}CH(O\cdot CO\,CH_3)CH_2CH:CH\cdot(CH_2)_7COOH \longrightarrow$

Acetylricinolein

$C_6H_{13}CH(O\cdot CO\cdot CH_3)CH_2CH-$

$+ \quad -CH(CH_2)_7COOH$

3) $C_6H_{13}CH(OH)\cdot CH_2CH_2(CH_2)_7COOH \longrightarrow$

1 2-Oxystearin

$C_6H_{13}CHO + CH_3CH_2CH(CH_2)_7COOH$

Undecylic acid.

Among the above mentioned products $\Delta 9$ undecyleic acid formed from ricinoleic acid is decidedly noxious as proved by the author in his former experiment,[1] while others from acetylricinolein and 12-oxystearin have no noxious effect at all.

It seems that the breaking of the molecules occurs at first, at the point where OH group is attached or where double linking is present.

Summary of the Results.

1) The nutritive value of the fats containing oxy-fatty acids differs according to the position of OH group. Thus, for instance, α- and 1 2-oxystearin were found to have quite different values.

2) The fats containing α-oxy acids are inferior to those of the corresponding saturated fatty acids, so it is improbable that α-oxydation occurs in the animal body.

3) The noxious effect of certain α-oxy acids decreases with the increase of the molecular weights. Thus for instance, α-oxyheptylin and α-oxymyristin have stronger toxicity than α-oxypalmitin or α-oxystearin.

4) The nutritive value of oxy-acids depends on the position of OH

group, rather than the number of OH groups in the molecule.

5) The nutritive value of ricinolein is greatly improved by acetylation, but as to dioxyundecylin, dioxystearin and trioxystearin, the acetylation had little effect upon the nutritive value.

6) The improvement of the nutritive value of ricinolein by acetylation may be due to the formation of different products by breaking of its molecule.

7) Ricinolein was found to be better than its solid isomer, ricinoelaidin.

ON THE DISTRIBUTION OF A NEW THIOAMINO-ACID.

by Satoru Ohdake.

(Abstract from the Original Paper)

(Received Sept 4th., 1926.)

In 1924, U. Suzuki, T. Mori and the author isolated a new sulphur compound from the alcoholic extract of yeast, and gave the empirical formula $C_{11}H_{15}NSO_3$ to it. Boiled with diluted acids, it was hydrolysed easily to Adenin ($C_5H_5N_5$) and a new thiosugar ($C_6H_{12}SO_4$): so the authors concluded, this compound should be adenyl–thiomethyl–pentose. (U. Suzuki, S. Ohdake, and T. Mori:- The Journ. of the Agricultural Chemical Society of Japan. Vol. I No. 2 p. 127–136, 1924 and Biochemische Zeitschrift, B. 154, Heft. 3/6 S. 278–289, 1924.)

On studying further the alcoholic extract of yeast, the author isolated a new thioamino–acid in the following way:- The alcoholic extract of yeast was evaporated under diminished pressure to a syrupy consistence and dissolved in a little water. A concentrated tannin solution was then added, the precipitate thus formed, was collected, decomposed with baryta water and filtered. The filtrate, freed from an excess of baryta, was evaporated to a small volume, when the crystals of adenyl–thiomethyl–pentose separated out which were filtered off. To this filtrate, strong alcohol was added enough to make the alcoholic content of the mixture 80% by volume. The voluminous precipitate thus formed, was filtered by suction, and recrystallised several times from diluted alcohol. The crystals were found to be the mixture

of leucin and a sulphur compound, but it was impossible to separate them by fractional crystallisation. It was therefore dissolved again in water, and the saturated solution of mercuric chloride was added. The sulphur compound alone, forming an insoluble salt with it, was precipitated and then decomposed with hydrogen sulphide. This treatment was repeated again and the resulted crystals were recrystallised from diluted alcohol. The yield of the purified compound was 0.6 g. from 334,280 kg. of fresh yeast.

The sulphur compound thus obtained having the emperical formula $C_5H_{11}SNO_2$ is apparently a thioamino-acid as the analytical results of the free compound as well as of its derivatives show.

The purified compound is colourless and crystallises in thin monoclinic plates. Heated in a capillary, it melts at 271-272°C (uncorr.) with decomposition. It is easily soluble in water and in diluted alcohol, but insoluble in ether, benzene etc. Its specific rotatory power is $[\alpha]_D^{16} = -11.77°$ in water. The aqueous solution of this compound gives a violet colour reaction with ninhydrin when warmed, but Millon's, Folin's, and biuret-reaction are all absent. With mercuric chloride, mercuric nitrate, and mercuric sulphate, it gives a white precipitate, but it is precipitated neither by phosphotungstic acid nor by picric acid. Even a boiling strong alkali does not split sulphur from this. compound in a form, detectable by sodium nitroprusside or by lead acetate, while both these reactions are positive, when it is fused with metallic sodium. In contrary to ethyl-cystein this sulphur compound is quite stable against a boiling strong alkali ; giving neither ammonia nor ethyl-mercaptane. (Compare ; Brenzinger :- Zeitschrift f. physiol. Chem. XVI. 563, 1892. Neuberg und Mayer :- Zeitschrift. f. physiol. Chem. **44**, 489, 1905.)

The copper-salt Cu $(C_5H_{10}SNO_2)_2$ forms light blue thin monoclinic plates which are somewhat soluble in boiling water but almost insoluble in the cold. Its derivative of α-naphtyl-isocyanate $C_{16}H_{18}N_2SO_3$ crystallises in white long needles, melting at 187°C (uncorr). It is almost insoluble in water, ether etc., but dissolves easily in alcohol. Its β-naphtalene-sulpho-derivative $C_{15}H_{17}S_2NO_4$ forms white needles, melting at 204°C (uncorr).

From these properties, the compound should be a new thioamino-acid having the formula $(C_3H_7S) - CHNH_2COOH$, besides the known sulphur compounds as cystin, cystein, and taurin etc. (S. Ohdake :- the Journ. of the Agricultural Chemical Soc. of Japan. Vol. I. No. 8. 1925, and Biochemische Zeitschrift. Bd. 161 Heft 4/6, 1925.)

Recently J. H. Muller isolated a new sulphur compound $C_5H_{11}SNO_2$ from hydrolytic products of casein and egg-albumin. (J. H. Muller :- Journ. of Bact. VII p. 309-325. 1922 and Journ. of biol. chem. LVI. No. 1. 1923).

The thioamino-acid, isolated by the author from the yeast-extract, has entirely the same properties with it. For the identification of these two substances, the author prepared the sulphur compound from casein according to Muller's mercuric method :-

Casein was hydrolysed with sulphuric acid, and neutralised with sodium hydroxide solution. To the filtrate, the sulphuric acid solution of mercuric sulphate was added, and the mixture was again neutralised with caustic soda. The precipitate was then extracted with hot 2 % baryta water and filtered by suction. The filtrate freed from mercury and barium was evaporated to a small volume. Then the boiling saturated solution of mercuric chloride was added, using about 30 g. of the reagent for each pound of protein. Standing about 30 hrs., the precipitate was collected and decomposed with hydrogen sulphide. The filtrate freed from mercury was evaporated to dryness in vacuo, and the residual substance was dissolved in water and treated with freshly prepared silver oxide. The silver chloride is filtered off and the excess of silver was removed with hydrogen sulphide. The filtrate from silver sulphide was evaporated to a small volume in vacuo. Finally, three or four volumes of hot alcohol were added to it, and on cooling, the sulphur compound separated as shining crystals. For the purpose of the purification, these crystals were dissolved in about ten times of hot water and treated with mercuric chloride again as described above. The yield of the pure compound was 1.12-1.74 g. from each pound of casein.

As expected, the sulphur compound thus prepared from casein had entirely the same properties with that isolated from yeast-extract, except that its specific rotatory power was a little lower. It was proved that, the compound isolated by Muller was partially raceminized in the course of the extraction with hot baryta water.

The author, further, isolated the same thioamino-acid from the hydrolytic product of yeast-protein by the same treatment. It is clear, therefore, that the thioamino-acid isolated from yeast-extract must have been produced by the autolysis of yeast itself.

Although the existence of non-cystine-protein-sulphur has already been suspected often, it was still an open question if there exist one or more sulphur compounds in protein molecule other than cystin. The isolation of new sulphur compounds from protein should be a sole key to solve this problem.

To know the distribution of the "thioamino-acid," the author worked on the several kinds of protein by the same mercuric method as described above, and isolated the same thio-compound in a pure state though not quantitative.

The yields of the "thioamino-acid" are as follows :-

Casin	0.25-0.39 %
Egg-albumin "Merck"	0.42 %
Blood-fibrin	0.26 %
Beer-yeast	0.008%
Protein from rice-bran	0.002%
Protein from soya-fean	——

From these results, the author has come to the general conclusion that the "thioamino-acid" is very widely distributed and is comparatively abundant in amimal proteins but scanty or even absent in vegetable proteins, especially in so-called reserve-proteins.

The author wishes to express his sincere thanks to Prof. Dr. U. Suzuki for his kind guidance.

ON THE SAPONIN OF ADZUKI BEAN.

By E. Takahashi and K. Shirahama.

(Received Aug. 26th, 1926.)

Isolation and properties : Pulverized Adzuki-bean was extracted with 95% alcohol and the extraction was evaporated to dryness after the magnesium oxide had been added.

The residue was again extracted with alcohol and the saponin was precipitated with ether from the solution.

By the repeated precipitation as above, the pure saponin was obtained as a amorphous yellow powder.

The substance was soluble in water, alcohol or phenol and its aqueous solution foamed markedly on shaking and with concentrated sulphuric acid it gave a characteristic reddish purple coloration.

Elementary analysis gave the following results.

(1) 3.580mg. saponin gave 6.355mg. CO_2, 2.250mg. H_2O

(2) 4.147mg. saponin gave 7.318mg. CO_2, 2.540mg. H_2O

	(1)	(2)	Average
C.	48.41%	47.86%	48.13%
H	7.03%	6.82%	6.93%

Haemolysis : Haemolytic property was tested with red blood corpuscles of an ox. The washed corpuscles were mixed with physiological saline solution of saponin and kept one hour for 37°C. Haemolysis took place in 1.5% saponin solution.

Haemolysis : Pure saponin was boiled with 1 % sulphuric acid for 18 hours, by which the saponin hydrolysed into prosapogenin and a sugar, glucose.

By further hydrolysis with 6% sulphuric acid for 5 hours the prosapogenin was decomposed into sapogenin and arabinose.

By the estimation of these sugars, the quantitative ratio among sapogenin, arabinose and glucose was determined as 1 : 1 : 16.

The sapogenin was white amorphous powder insoluble in water, soluble in alcohole and phenol.

It was very stable for acid and alkali.

(1) 4.425mg. sample gave 10.820mg. CO_2, 3.900mg. H_2O

(2) 2.925mg. sample gave 7.170mg. CO_2, 3.050mg. H_2O.

	(1)	(2)	Average
C	66.69%	66.85%	66.70%
H	10.86%	11.02%	10.90%
O	—	—	23.40%

From the above data and by the measuring of the freezing point depression of its phenol solution, its molecular formula was calculated as $C_{23}H_{45}O_6$.

So the molecular formula of Adzuki-saponin is determined as $C_{23}H_{45}O_6$. $C_5H_8O_4$. $16C_6H_{10}O_5$.

Discussion : Power and Salway isolated a kind of saponin from the root of a species of Adzuki, Phaseolus Multiflora (Pharm. Journ. p. 553, 1913) and gave the formula $C_{26}H_{44}O_4$ to the sapogenin, and $C_{57}H_{94}O_{24}$ to the saponin. They isolated rhamnose by hydrolysis of the saponin and the ratio of sapogenin to rhamnose was determined as 1 : 4.

But the saponin isolated by us, appears to be quite different from that of Power and Salway. It in composed of a sapogenin, arabinose and glucose in the ratio 1 : 1 : 16. The difference is probably due to the difference in the species of the plant. It is also conceivable that substance in the roots undergoes change during the removal to the bean.

A NEW METHOD FOR QUANTITATIVE ESTIMATION OF STARCH BY ASPERGILLUS AMYLASE (TAKA-DIASTASE).

By Kokichi Oshima and Shin-ichi Itaya.

(Received Sept. 2nd., 1926)

The procedure of the new method is as follows :–

Mix 1 gram of powdered sample with 80c.c. water in a flask of hard glass and cook for 10 min. at 100°C in an autoclave or for 1 hour in a boiling water bath. Add a mixture of 3.2c.c. M/6 citric acid and 6.7c.c. M/6 Na_2HPO_4 to keep the whole liquid at pH 5.2. Further add 10c.c. of 3.0% apueous solution of Taka-diastase (made by Sankyo & Co. or Park and Davis & Co.) or a strong enzymic preparation obtained from Aspergillus oryzae and 1 c.c. of toluol. Shake and close well, and keep it for 24 hours at 40°C. After that period, dilute the contents to 200c.c. with water and filter it through dry filter paper. With 20c.c. of the filtrate, determine the reducing activity by Bertrand's or other methods and calculate the reducing matter as glucose. Then multiply the quantity with 0.9 to obtain starch quantity. Of course it is necessary to subtract the reducing matter present in the sample before digestion and that produced by autolysis of Taka-diastase used.

The following results were obtained by the preliminary experiments and by application of the new method.

1. The optimum reaction of the amylo-saccharifying action of enzyme obtained from Aspergillus oryzae (Ahlb.) Cohn is pH 2.5.

2. One hour's heating of the enzyme in about neutral solution at a temperature below 45°C has no effect upon the amylase. At 50°C the activity is a little reduced, at 55°C it is reduced nearly half and above 65°C all the enzymic activity is lost. More prolonged heating has greater destructive action, but at a temperature lower than 40°C at almost neutral reaction, the amylase undergoes no change, even after one year, provided it is protected from other injurious action.

3. At pH 4.5 for one hour at room temperature, destruction of the amylase begins, and at pH 2.5 it is almost entirely destroyed ; at pH 8.4 its activity also begins to decrease and at pH 10 almost all the activity is lost.

4. For heating the amylase solution, pH 6.4 is the most stable reaction.

5. The final decomposition product of starch by Asp. amylase is recognized to be glucose exclusively. However, if the quantity of the amylase used is less than a certain minimum, the glucose production is not complete even though the digesting period is prolonged.

6. For the cooking of starch samples, one hour in boiling water has almost same effect with cooking for 10 min. at 110°C in an autoclave.

7. Comparison of starch content in many kinds of cereals, roots and their products determined by the new method and by the decomposition with HCl is as follows :–

Starch Content in Dry Matter of Samples.

Sample	Moisture(%)	Starch (%)	
		By new method	By HCl method
Soluble starch	10	98.40	99.16
Dry potatoes	10	77.60	73.20
Dry sweet potatoes	15	71.78	75.39
Unpolished rice	10	71.30	72.40
Kaoliang	13	69.51	71.28
Mais	11	67.24	70.66
Starch residue	15	65.54	68.61
Barley	15	63.10	68.61
Italian millet	13	50.37	56.16
Oat	15	44.47	61.41
Wheat bran	13	28.35	46.13
Rice bran	11	16.96	30.84

The above results show that there is a big difference of starch content in oat, wheat bran and rice bran etc. These starch contents by the new method are much alike with that obtained by the method of malt diastase on which Sherman and others have reported.

8. The result of the alcohol fermentation of these samples corresponds well with the starch content determined by the new method.

9. This new method is more accurate than the HCl method and simpler and easier than the malt diastase method.

(Hokkaido Imperial University. August 1926)

THE NATURE OF THE ACIDITY OF MINERAL-SOIL.

(*Abstract.*).

By Shigeru Osugi and Yoshio Sano.

(*Department of Agriculture, Kyoto Imperial University, Kyoto, Japan.*)

(Received Sept. 9th., 1926.)

1. The mineral-acid-soils are able to invert cane sugar to a remarkable extent and this inverting action is not due to the presence of any soluble acid substance in the soil but to the surface action of soil-particles; and from many experimental results, it is ascribed to the action of acid aluminium silicate in the soil.

2. The hydrogen ion concentration of the water extract of the soil is not high enough to explain the inverting action of soil.

It was proved that the higher proportion of soil to water (1/3–1/50) gives the higher hydrogen ion concentration in the extract, (0.05–0.5 as Ph.) and that the extract of clay part (<0.01m.m.) has the higher concentration of hydrogen ions (0.2–1.5 as pH.) than that of coarser part (<0.5m.m.) of the same soil.

From these results, it is reasonably expected the hydrogen ion concentration in the absorbed water film around soil-particles, especially of the clay part, to be considerably higher than that in the surrounding liquid, and indeed, so high that it inverts cane sugar so distinctly as mentioned above.

3. Shaking the soil at a room temperature and at 90–100°C. accelerates the inversion reaction (5–40%) and this shows again that the reaction takes place mainly on the surface of soil-particles.

4. Grinding the soil-particles decreases the concentration of the hydrogen ions in the extract (about 0.1 as pH.) and diminishes the inversion reaction (8–35%), and repeated freezing and thawing the soil increases both, (0.02–0.28 as pH. and 6–34% of inversion) but the conductivity of the extract increases in both cases. (7–29% by grinding and 5–36% by freezing)

From the above results, it is concluded that the inversion reation occurs only on the surface of soil-particles having certain hydrogen ion concentration, and only on the insoluble acid or acid substance in soil.

5. The mineral-acid-soils, air-dried and even dried at 90–100°C can invert cane sugar in a glycerine solution (5% sugar disolved in glycerine free from water) and this shows again that the inversion reaction should occur

only on the surface of soil-particles.

6. When any weak alkali-solution is added to the soil suspension, one of the following changes in the conductance should take place according to the property of the suspension.

(1). When the suspension has no action upon the solution, the conductivity of the solution suffers no change.

(2). When the suspension adsorbs the base in the solution physically, the conductivity should decrease.

(3). When the ssupension is of acid-nature and combines chemically with base, the conductivity should increase.

And when a strong alkali-solution is added to the suspension of acid-nature, the conductivity should decrease.

A test was made with the acid-soil and ammonia solution, and the increase of the conductivity was noted (30–96%) but with sodium hydroxide solution, the distinct decrease was observed. (30–90%)

From these experiments, it is shown that the substance causing the acidity in the soil is of true acid-nature.

7. An experiment on the influence of heating the soil shows that the heating of two hours at 80°, 90°, and 100°C. causes no change upon the inverting action and the degree of acidity (hydrolytic and the exchange) but at 250°C., the former begins to diminish and at 550°C., it almost disappears.

Bouyoucos has recently carried out experiments on the effect of heating upon the physical properties of soil and reported that the heat of wetting, the unfree water and the plasticity of soil, begin to diminish at 230°C. and at 485°C., these properties are almost dissipated.

The results shows that the inverting action of the soil and the physical properties named above are closely correlated, and that the substance causing the inverting action of the soil is colloidal.

STUDIES ON PROTEINS IV.
ON THE PREPARATION OF RICE-GLUTELIN.

(Contribution No. 5 from the Laboratory of Nutritional Chemistry, Dept. of Agriculture, Kyoto Imperial University)

By

Kinsuke KONDO and Tunetomo HAYASHI.

(Received Sept 8th., 1926.)

A. In great probability it is impossible to prepare such a chemically pure single protein as we desire. This point was elucidated by Sörensen's experiments.(1) As is well known, we can find in polished rice besides other constituents four kinds of protein such as albumin, globulin, glutelin and prolamin. We may reasonably assume these proteins occur in the rice itself, as well as in the special protein fractions obtained from it, not as mixtures of proteins, but as their combinations, as Sörensen considers in regard to serum-globulins. Consequently even tho we might isolate a special protein fraction from the natural substance, this fraction could not consist of merely the desired single protein. We state herewith that it is a reasonable question to-day whether the fraction thus obtained is reproduceable or not. Presumably it would be secondary whether pure or not. However we may expect to prepare a well-defined and reproduceable protein. In studying the chemistry of proteins we shall be satisfied at present with such a protein as discussed above.

B. a. Rice-glutelin, as well as other kinds of proteins, capable of change in an alkali-solution, even tho this be not very strong. In preparing the rice-glutelin we may first remove the albumin and globulin from the rice-powder. For this purpose we extract the rice powder with a 10% NaCl solution 3 times continuously and then wash out completely till it contains no NaCl.

The residue is mixed with water and then with a 0.05n NaOH solution by means of dropping in such a manner that the concentration of NaOH will be under 0.025n. Then the rice-glutelin dissolves into the NaOH solution. This solution is clarified and freed from starch particles and other ingredients after repeated filtration. Into the clear solution thus obtained a

(1) Comptes-rendus du Lab. Carlsberg **12**. (1917)
Zeitschr, physiol. Chem. **103**. (1918)
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certain amount of 0.05n acetic acid is dropped till the glutelin flocculates out as completely as possible. The glutelin precipitate is filtered and washed respectively and then redissolved and reflocculated. Such a procedure is repeated 3 times continuoulsy to make it free from other nitrogenous ingredients, and this was proved experimentally. In this whole treatment about one third of the nitrogenous substance in rice powder is lost.

B. b. We purified our rice-glutelin mineral substance and acetic acid by application of dialysis. The experiments show that the rice-glutelin becomes practically entirely free from any diffusible ingredient, such as mineral matter, in 6 or 7 days. Hence, we can believe that the protein thus purified contains practically no other ingredient. We call it Rice-glutelin No. 1.

B. c. We made our rice-glutelin into anhydrous state with the help of alcohol and ether and determined the amount of nitrogen and its distribution by the usual method.

B. d. It is proved that a 0.5n NaCl solution is a more reasonable solvent for removing globulin from the rice powder than a 10% NaCl solution, which is used commonly. Hereafter we shall prefer a 0.5n NaCl solution in the foregoing preparation of glutelin to any other.

(Sept. 5, 1925)

STUDIES ON PROTEINS V. ON THE POINT OF OPTIMUM FLOCCULATION OF RICE-GLUTELIN.

(Contribution No. to from the Laboratory of Nutritional Chemistry, Dept. of Agriculture, Kyoto Imperial University).

By

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A. After Michaelis,[1] an iso-electric point of ampholyte difficult of solution, i. e. casein, falls on the point of an optimum flocculation.

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(1) Michaelis:- Biochem. Zeitschr. **47**, 250 u. 260. (1912)

The rice-glutelin is, like casein, an ampholyte difficult of solution. Hence the rice-glutelin must flocculate at a maximum if the reaction of the solution is to become an iso-electric point of the rice-glutelin. But even if our rice-glutelin successively flocculates and precipitates at a maximum in appearance a part of the protein remains in the dissolved from. Therefore we may reach a conclusion as to whether the reaction of the solution is apart from the iso-electric point of our rice-glutelin or whether the so-called optimum or maximum flocculation does not mean the perfect flocculation. We will in the present work examine the point of flocculation of our rice-glutelin and study its condition in the state of optimum flocculation.

B. We examined the point of optimum flocculation of Rice-glutelin No. 1 in acetate-acetic acid mixtures. We find that this protein can flocculate and precipitate at a maximum in the diluted sodium acetate solution, whose reaction is near absolute neutrality.

And our experimental results show that the more diluted the sodium acetate solution becomes, the farther the reaction goes towards the iso-electric reaction of this protein.

But the rice-glutelin can not completely flocculate even at the point of optimum flocculation, and the latter is changeable according to the presence of any salt in the solution. This was elucidated by means of acetate solutions and Sörensen's phosphate mixture. We find also that the reaction of the solution, in which the protein can flocculate at a maximum, changes after the addition of the protein.

C. We searched for the cause of this in the difference of the protein-ionizing powers of the ions derived from the salt, beside the hydrogen ion activity. And many salts have such a property. Hence the protein can not completely take form of $[R<^{NH_2}_{COOH}]$ in the presence of salt in the solution. In other words, the protein can not behave as an iso-electric ion in the presence of salt in the solution.

Applying these facts and considerations to the theory on the iso-electric point, we conclude it is impossible to determine the iso-electric point of a protein difficult of solution, such as casein and rice-glutelin, by the usual method. Hence we define the point, determined by this last, as an apparent iso-electric point and differenciate it from a theoretical one. But for a protein such as native albumin, whose iso-electric reaction is independent of the presence of salt, the apparent and theoretical iso-electric points are identical with each other.

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